

Initiation of Autoimmunity by a Reactive Metabolite of a Lupus-Inducing Drug in the Thymus

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Drug-induced lupus is a side effect of deliberate ingestion of various medications, but its etiology, underlying mechanisms, and pathogenesis are puzzling. *In vivo* metabolic transformation of lupus-inducing drugs to reactive products explains how a heterogeneous set of drugs can mediate the same disease syndrome. Evidence has accumulated that drugs are transformed by extracellular oxidation from reactive oxygen species and myeloperoxidase produced when neutrophils are activated, maximizing the *in situ* accumulation of reactive drug metabolites within lymphoid compartments. The metabolite of procainamide, procainamide hydroxylamine, displays diverse biologic properties, but no apparent autoimmune effect has been observed. However, when procainamide hydroxylamine was introduced into the thymus of young adult normal mice, a delayed but robust autoimmune response developed. Disruption of central T-cell tolerance by intrathymic procainamide hydroxylamine resulted in the production of chromatin-reactive T cells that apparently drove the autoantibody response in the periphery. Drug-induced autoantibodies in this mouse model were remarkably similar to those in patients with procainamide-induced lupus. Therefore, this system has considerable promise to provide insight into the initiating events in drug-induced lupus and may provide a paradigm for how other xenobiotics might induce systemic autoimmunity. **Key words:** autoantibodies, drug-induced, lupus, T cells, reactive drug metabolites, thymus. — *Environ Health Perspect* 107(suppl 5):803–806 (1999).

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It has been known since the initial description almost five decades ago of a side effect of long-term medication with hydralazine (1,2) that certain drugs have a propensity for inducing autoantibodies and occasionally lupuslike symptoms. Over the years some 46 drugs have been implicated with various degrees of risk in the induction of lupuslike disease, 38 of which are currently in use. Although drug-induced lupus is not a major clinical problem because it can be fully cured by discontinuing use of the medication, it is remarkable that a low molecular weight xenobiotic has capacity to initiate or sustain a well-defined systemic autoimmune disease. Not only might this phenomenon shed light on the process of self-/non-self-discrimination that normally operates to avoid autoimmunity, but it may offer a paradigm on how other xenobiotics disrupt the immune system. Drug-induced lupus occurs in approximately 20% of patients treated with the antiarrhythmic drug procainamide for 1–2 years, and almost all procainamide-treated patients eventually develop particular autoantibodies, whether or not lupuslike symptoms appear. From drugs such as procainamide, hydralazine, and quinidine, which have a rather high propensity of autoimmune manifestations in response to exposure, drug-induced lupus has become the best-documented example of an environmentally induced autoimmune disease due to a noninfectious agent.

The symptomatology of drug-induced lupus and its objective features such as autoantibodies

to chromatin-derived antigens are remarkably similar to those of systemic lupus erythematosus, a disease that arises spontaneously. Drug-induced lupus does not behave like a hypersensitivity reaction to a drug-altered self-antigen because of its slow kinetics (delay of months to years from the onset of drug therapy to the development of autoantibodies and clinical symptoms), correlation with drug dose, and the inconsistent recurrence of symptoms upon drug rechallenge. In contrast, drug hypersensitivity reactions are characteristically drug dose independent and recur immediately after rechallenge with the inciting agent (3). Furthermore, as discussed below, lupus-inducing drugs are heterogeneous, with no common structural properties, specific affinity for the targets of autoantibodies, or capacity to enhance or inhibit autoantibody binding.

In trying to understand the mechanism underlying this phenomenon, it is important to recognize that a wide range of therapeutic purposes are encompassed by drugs having lupus-inducing potential. Although some of these drugs are aromatic amines, there is no pharmacologic, chemical, or therapeutic feature that links drugs with the capacity to induce lupuslike disease. Yet symptoms of arthralgias, myalgias, and constitutional symptoms such as fever, weight loss, and fatigue are generally not drug specific, and the specificities of autoantibodies induced are essentially the same regardless of the inciting agent. Therefore, it is likely that the underlying immune perturbations responsible for lupus induced by all drugs are

similar, leaving as enigmatic how a heterogeneous collection of xenobiotics can act through an apparently common mechanism.

Neutrophil-Mediated Metabolism of Lupus-Inducing Drugs

Drug-induced lupus is a type B adverse drug reaction (4) that cannot be predicted from the drug's known pharmacology. One way such idiosyncratic drug reactions can occur is through the *in vivo* generation of drug metabolites displaying new properties. Hepatic drug metabolism is well established, and the phase I reactions mediated by the microsomal monooxygenase system and certain esterases can produce reactive compounds substantially different from the parent drug. Incubation of procainamide with human or rat liver microsomes that contain the mixed-function oxidases results in the formation of an unstable product, procainamide hydroxylamine (PAHA) (5,6). PAHA can be detected after the perfusion of rat liver with procainamide in a blood-free environment (7). Hydralazine and isoniazid are also susceptible to hepatic oxidative metabolism (8,9). PAHA is taken up by erythrocytes, and oxyhemoglobin enhances its biologic activity (7,10), presumably by converting PAHA to nitroso-procainamide (11,12). Together with the demonstration that rats treated with procainamide had increased liver lipid peroxide levels and antioxidant activity (13), hepatic metabolism of lupus-inducing drugs demonstrates that their chemistry is compatible with the *in vivo* generation of reactive drug metabolites with potentially new biologic properties.

Despite the probable importance of drug metabolism in the etiology of drug-induced lupus, it is doubtful that liver-mediated drug transformation is important for the following reasons: a) hepatotoxicity is not associated with

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drug-induced lupus; *b*) liver-generated xenobiotic metabolites typically bind to microsomes or macromolecules near their site of formation and fail to be produced in sufficient amounts to interact with *in situ* lymphocytes or antigen-presenting cells or to exit the liver in reactive forms; *c*) although drug metabolites might bind to self-antigen in the liver, lupus-inducing drugs are very small and diverse in chemical structure, with little common structural information to specifically interact with the chromatin-derived targets that characterize the autoimmune response in drug-induced lupus (see "Role of T Cells in Drug-Induced Lupus"). Therefore, if *in vivo* metabolism is a prerequisite for initiating drug-induced lupus, an extrahepatic process may be involved in which reactive metabolites are generated within an immune compartment where they can act on the cellular elements of the immune system to prevent or break tolerance.

Using procainamide as the prototype, we were the first to report that activated peripheral blood neutrophils have capacity to metabolize procainamide to PAHA (14); the role of the respiratory burst and degranulation events associated with neutrophil activation was confirmed (15) and analyzed in detail (16). Drug metabolism by neutrophils occurs in the extracellular environment, in which released myeloperoxidase, acting with hydrogen peroxide produced upon neutrophil activation, enzymatically oxidizes copresent drugs (17). All major pharmacologic classes of lupus-inducing drugs have the capacity to undergo transformation to reactive products by exposure to activated neutrophils (17–21). There is a general correlation between susceptibility to neutrophil-mediated transformation and propensity to induce lupus (17); chemical analogs of each drug such as the non-lupus-inducing analog of procainamide, *N*-acetylprocainamide, were resistant to metabolic transformation by this mechanism.

Metabolism of drugs by activated neutrophils provides a mechanism for generating highly reactive metabolites directly within lymphoid tissue, where autoimmunity presumably develops. Because neutrophils are present in high concentration in the circulation, local production of labile compounds can potentially occur in any lymphoid compartment where neutrophils have access. This process would minimize dilution, time-dependent hydrolysis, and circulatory distribution of reactive metabolites, thereby maximizing their biologic effect on the tolerance status of lymphocytes.

Biologic Properties of Reactive Metabolites of Lupus-Inducing Drugs

Reactive intermediates of procainamide (11,22), hydralazine (23–25), chlorpromazine (18), and isoniazid (9) show capacity to covalently bind proteins nonspecifically and

kill a wide variety of cells under certain *in vitro* conditions at pharmacologically relevant concentrations (10,12,17,26). PAHA has also been reported to be slightly mitogenic for human lymphocytes (10). Other biologic properties have been demonstrated for PAHA including enhancing reactive oxygen species production by murine macrophage (27) and human neutrophils (10) and generating PAHA-specific murine T cells *in vivo* (28). The chemical reactivity, cytotoxicity, and immunogenicity of PAHA reflect the highly reactive nature of drug metabolites in general; however, none of these effects can be directly related to the singular property of these drugs to induce autoimmunity, as none of the aforementioned biologic effects of drug metabolites include autoimmune features. In addition, *in vivo* administration of PAHA into the spleen or in other peripheral sites and adoptive transfer of syngeneic splenocytes treated *ex vivo* with PAHA failed to induce autoantibodies.

Role of T Cells in Drug-Induced Lupus

The hallmark of drug-induced lupus is the appearance of autoantibodies to the (H2A-H2B)-DNA subunit of chromatin. However, just as with protective immunity, appearance of such IgG antibodies to self requires the participation of cognate T-cell help. T cells are generated in the thymus and are normally tolerant to self because differentiation and development of T cells in the thymus is linked with a process that prevents autoreactivity—central T-cell tolerance. In contrast, peripheral tolerance refers to other mechanisms employed by T cells for avoiding autoreactivity after they develop to maturity in the thymus. For example, when peripheral T cells become activated upon recognition of an antigen in the form of a peptide bound to the major histocompatibility complex, they undergo clonal expansion. However, the extent of cell division is limited by a process called activation-induced cell death, which can be a consequence of enhanced expression of the membrane protein Fas and its ligand whose interaction triggers apoptosis. As a result, most of the progeny of

the T cell disappear, and it is generally believed that autoreactive T cells that happen to avoid central cell tolerance are usually killed by this peripheral tolerance mechanism.

Conceptually, autoimmunity is the converse of immune self-tolerance, so if drugs reverse ("break") tolerance of mature lymphocytes or prevent establishment of self-tolerance during lymphocyte development, autoimmunity might be the consequence. Therefore, lupus-inducing drugs could act either by breaking tolerance of peripheral T cells to self-antigens or preventing acquisition of self-tolerance during T-cell development in the thymus. Breaking peripheral T-cell tolerance occurs upon *in vitro* treatment of syngeneic-activated splenocytes, resulting in an interesting lupuslike pathology including autoantibodies and glomerulonephritis (29,30). These phenomena appear similar to the autoimmune side effects accompanying the graft-versus-host reaction upon adoptive transfer of semiallogeneic T cells (31). However, drug-induced lupus has much more limited hyperimmune features than the global perturbations characteristic of a graft-versus-host reaction, suggesting that if loss of peripheral T-cell tolerance is involved in drug-induced lupus, it is operating on a susceptible or primed population of autoreactive T cells that arose by some other mechanism.

The possibility that failure of central T-cell tolerance might initiate drug-induced lupus has been generally ignored because it is widely assumed that the bulk of the T-cell repertoire is created before birth or soon thereafter. In contrast, drug-induced lupus typically occurs in individuals > 50 years of age, the age group most likely to be treated with drugs with a propensity to induce lupus. However, a systematic reevaluation of thymus structure during aging has revealed that thymic involution and replacement of lymphocytic with adipose tissue starts soon after birth and continues at a steady rate of only approximately 3% per year until middle age, when loss of lymphoid tissue slows down to < 1% a year (32). As summarized in Table 1,

Table 1. Evidence for thymic function in the adult.

	Structural	Functional
Mouse	Lymphocytic islands in the aged thymus exhibit a T-cell subset distribution similar to the young thymus (33)	Reconstitution of the V β repertoire after bone marrow transplantation in irradiated old mice (34) Production of mature T cells <i>de novo</i> from mice >1 year of age (35) Positive and negative selection were retained despite thymic involution (36)
Human	Lymphocytic islands in the aged thymus exhibit a T-cell subset distribution similar to the young thymus (37) DNA excision circles characteristic of newly formed T cells are detected in the circulation only in nonthymectomized elderly people (39).	Naïve T-cell subsets are generated <i>de novo</i> from transplanted bone marrow or peripheral stem cells after myelosuppression (38)

there is a growing body of evidence in the mouse and human that the thymus functions in the adult to produce mature T cells. Therefore, the machinery for preserving T-cell tolerance by this central immune organ must be maintained throughout life, and interference with this process by drugs could result in autoimmunity.

Disruption of Central T-Cell Tolerance Causes Lupus-Like Autoimmunity

To determine whether disruption of central T-cell tolerance by lupus-inducing drugs could result in autoimmunity, PAHA was injected into the thymus of young adult (C57BL/6 × DBA/2)F₁ mice. Two intrathymic injections with the drug resulted in the rapid appearance of IgM anti-denatured DNA antibodies, followed by a delayed but sustained production of IgG antichromatin antibodies (Figure 1) (40). Specificity, inhibition, and blocking studies demonstrated that the serology of PAHA-treated mice had a striking resemblance to that of patients with procainamide-induced lupus, suggesting that the immune system had undergone similar perturbations in both species (Figure 2). In the peripheral immune organs such as the spleen and lymph nodes of PAHA-treated mice, chromatin-reactive T cells were detected at a time point when antichromatin antibodies

started to rise (40), suggesting that PAHA action in the thymus resulted in the export of autoreactive T cells to the periphery where they provided T-helper cell function to B cells with potential to produce autoantibodies.

Adoptive transfer of PAHA-induced chromatin-reactive T cells into otherwise untreated mice was sufficient to induce an autoantibody response (41). However, relatively large numbers of chromatin-reactive T cells were required to elicit an IgG antichromatin response, whereas adoptive transfer of less than 5 million T cells resulted in a much more restricted immune response (Table 2). These observations, together with the finding that two intrathymic injections were required to induce IgG antichromatin antibodies, suggested that peripheral tolerance mechanisms were limiting the capacity of chromatin-reactive T cells to provide helper function for B cells. Antichromatin antibodies developed after a single intrathymic PAHA injection into the Fas-deficient C57BL/6-*lpr/lpr* mice (41), suggesting that Fas-mediated activation-induced cell death was responsible for limiting autoimmunity in normal mice. However, as demonstrated with two intrathymic PAHA injections or by adoptive transfer of large numbers of chromatin-reactive T cells in Fas-intact mice, peripheral tolerance mechanisms fail to prevent autoimmunity in

normal mice when the immune system is subjected to a heavy load or second wave of chromatin-reactive T cells. Therefore, although defective activation-induced lymphocyte death (41) or drug-induced graft-versus-hostlike syndrome (29,30) might accelerate autoimmune disease, it appears that repetitive emigration of autoreactive T cells from the thymus is sufficient to initiate and sustain systemic autoimmunity.

Presently there is no evidence that drug-induced lupus in humans is initiated when central T-cell tolerance is disrupted by the action of reactive drug metabolites in the thymus. The most compelling argument that the thymus is the critical target of xenobiotic action is *a*) this organ is the only site in the mouse where PAHA presence initiates systemic autoimmunity and *b*) the specificity of PAHA-induced autoantibodies in the mouse is very similar to those in patients with drug-induced lupus. It is possible that

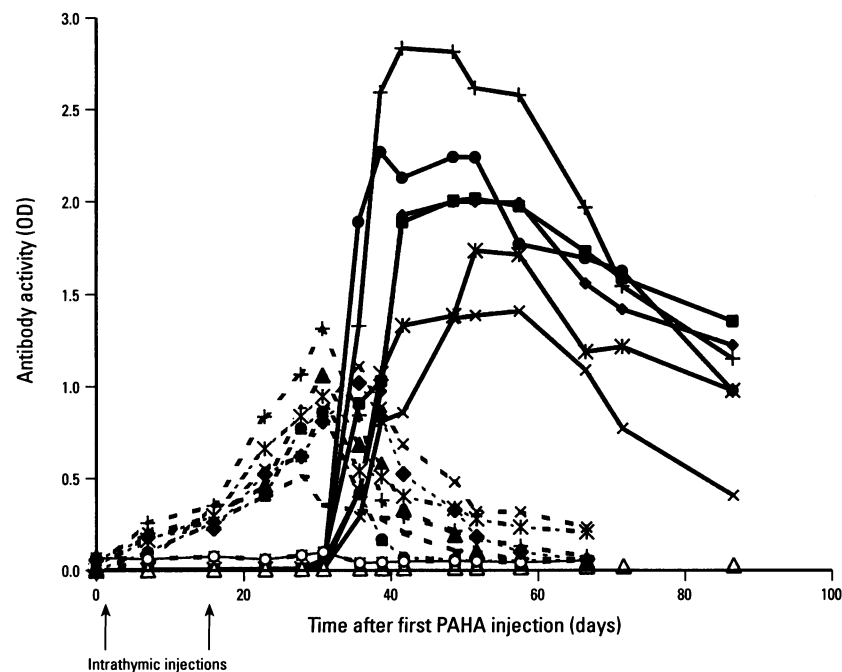


Figure 1. Serum autoantibody after intrathymic injection of PAHA. Abbreviations: anti-dDNA, anti-denatured DNA; OD, optical density; PAHA, procainamide hydroxylamine. Eleven (C57BL/6 × DBA/2)F₁ mice received two intrathymic injections of 4 mM PAHA 2 weeks apart. Seven control mice were injected with phosphate-buffered saline. IgG antibody activity to chromatin (solid lines) and IgM anti-dDNA antibody (dashed lines) were determined by enzyme-linked immunosorbent assay in serial serum samples of individual mice represented by unique symbols. IgM anti-dDNA activity in a typical control mouse is shown (open circles). Reprinted from Kretz-Rommel et al. (40), with permission of the *Journal of Clinical Investigation*.

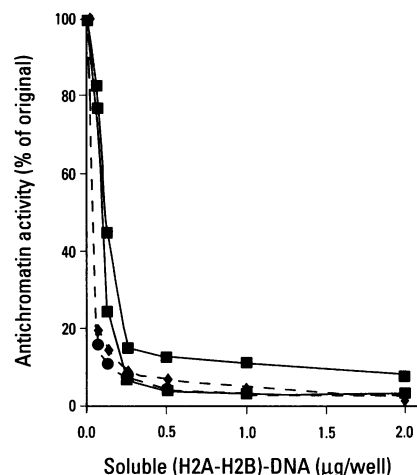


Figure 2. Inhibition of PAHA-induced antichromatin activity by (H2A-H2B)-DNA in a competition assay. Abbreviation: PAHA, procainamide hydroxylamine. The capacity to bind chromatin of IgG in 1:200 diluted sera of two (C57BL/6 × DBA/2)F₁ mice subjected to two intrathymic PAHA injections (—) or of two patients with procainamide-induced lupus (----) was measured in the presence of increasing amounts of soluble (H2A-H2B)-DNA. Reprinted from Kretz-Rommel et al. (40), with permission of the *Journal of Clinical Investigation*.

Table 2. Antibody activity in the serum of (C57BL/6 × DBA/2)F₁ mice after adoptive transfer of chromatin-reactive T cells.^a

Number of T cells transferred (3 mice/group)	IgM anti-dDNA at 1 week (mean OD ± SD)	IgG antichromatin at 3 weeks (mean OD ± SD)
500	0.189 ± 0.074	0
5,000	0.575 ± 0.450	0
50,000	0.618 ± 0.302	0
500,000	0.347 ± 0.143	0.201 ± 0.348
5,000,000	0.963 ± 0.738	0.324 ± 0.057

Abbreviations: anti-dDNA, anti-denatured DNA; OD, optical density; SD, standard deviation. ^aData from Kretz-Rommel et al. (41).

people with more active thymic function are particularly vulnerable to disruption of central cell tolerance by reactive drug metabolites, and demonstration of such a correlation should add considerable weight to the importance of this mechanism.

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